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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: M. Rigdon Lentz

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For: *METHOD AND COMPOSITIONS FOR TREATMENT OF CANCERS*

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. §1.132

I, M. Rigdon Lentz, hereby declare that:

1. I am a medical doctor specializing in the treatment of cancer patients.

2. A procedure is described in case report form, that utilizes apheresis and immunological affinity chromatography to treat a melanoma patient with short term need and weakening long term prognosis.

3. Innate, natural and antigen specific killer mechanisms represent our best arsenal for dealing with melanoma cells *in vitro* and *in vivo*. Central to these cellular destructive mechanisms is tumor necrosis factor (TNF-), an inflammatory cytokine produced by macrophages and earlier mononuclear cells and TNF- , a related cytokine produced and secreted by killerT-lymphocytes with highly selective antigen specific receptors, Old L.J., Antitumor activity of microbial products and tumor necrosis factor, and Bonavida B, et al., (eds): Tumor Necrosis Factor/Cachecin and Related Cytokines, Basell, Karger, 1988. p7; Haranaka K., et al, Cytotoxic activity of tumor necrosis factor (TNF) on human cancer cells in vitro, *Jpn J Exp Med* 1981; 51:191; Urban J.L.II, et al., Tumor necrosis factor: A potent effector molecule for tumor cell killing by activated macrophages, *Proc Natl Acad Sce* USA 1986; 83-5233; Philip R., et al., Tumor necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, Gamma-interferon and Interleukin-1, *Nature* 1986; 323:86; Ziegler-Heitbrock H.W., et al., Tumor necrosis factor as effector molecule in monocyte-mediated cytotoxicity, *Cancer Res*

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1986; 46:5947; and Feinman R., et al., Tumor necrosis factor is a important mediator of tumor cell killing by human monocytes, *J Immunol* 1987; 138:635. They derive from billions of clones, each with its own specificity. Thus, one clone of these thymus derived lymphocytes gives rise to T-killer (cytotoxic lymphocytes), or other functional classes each with the one specificity of the parent clone. Their mechanisms are related to both antibody dependent and antibody independent cellular tumor toxicity. Receptors for TNF on neoplastic, viral infected, aged cells or those otherwise targeted for destruction can be both a blessing and a curse. In a positive role, they allow binding of TNF to the surface for internalization and destruction of the cell. Unfortunately this receptor hypothesis has a double edge. Certain neoplastic cells such as active melanomas secrete large amounts of these receptors (sTNF-R1 and sTNF-R2) that promptly bind TNF before it can get within the vicinity of the cell, Haranaka K., et al, Cytotoxic activity of tumor necrosis factor (TNF) on human cancer cells in vitro, *Jpn J Exp Med* 1981; 51:191; Urban J.L.II, et al., Tumor necrosis factor: A potent effector molecule for tumor cell killing by activated macrophages, *Proc Natl Acad Sce* USA 1986; 83-5233; Philip R., et al., Tumor necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, Gamma-interferon and Interleukin-1, *Nature* 1986; 323:86; Ziegler-Heitbrock H.W., et al., Tumor necrosis factor as effector molecule in monocyte-mediated cytotoxicity, *Cancer Res* 1986; 46:5947; and Feinman R., et al., Tumor necrosis factor is a important mediator of tumor cell killing by human monocytes, *J Immunol* 1987; 138:635. This serves as a defense mechanism on the part of the targeted cell rendering the host immune system ineffective. TNF-R1 and R2 have been characterized with respect to molecular weights (55 and 75 kD respectively), Old L.J., Antitumor activity of microbial products and tumor necrosis factor, and Bonavida B, et al., (eds): Tumor Necrosis Factor/Cachecin and Related Cytokines, Basell, Karger, 1988. p7, Langkopf F., et al., Soluble tumor necrosis factor receptors as prognostic factors in cancer patients, *Lancet* 1994; 344:57-58; Howard S.T., et al., Vaccinia virus homologues of the Shope fibroma virus inverted terminal repeat proteins and a discontinuous ORF related to the tumor necrosis factor receptor family, *Virology* 1991; 180:633-664; Mathias S, et al., Activation of the Sphingomyelin signaling pathway intact EL4 cells and in a cell-free system by IL-lb, *Science* 1993; 259-519-522; and Andrews J.S., et al., Characterization of

the receptor for tumor necrosis factor (TNF) and lymphotoxin LT) on human T lymphocytes: TNF and LT differ in their receptor binding properties and the induction of MHC class I proteins on a human CD4+ T cell hybridoma, *J Immunol* 1990;144:2582-2591. They serve to both down regulate the immune response in a normal fashion and overly suppress the immune response as stated above with respect to certain malignancies. They are particularly abundant, and at high level, in patients with melanoma.

4. My previous studies utilizing ultrafiltration, with selective pore sieving by passing patient's plasma through cartridges, have been shown to reduce sTNF-R1 and R2 levels. The period of this procedure seems to be of sufficient length to allow TNF to rebound and selectively produce apoptosis or membrane disarray of melanoma cells, Gatanaga T., et al., Identification of TNF-LT blocking factor(s) in the serum and ultrafiltrates of human cancer patients, *Lymphokine Res* 1990;9:225-9. Instead of using ultrafiltrate cartridges, this apheresis system was coupled to Sepharose® gel columns in parallel, one of which contained monoclonal human anti TNF-R1 and the second anti TNF-R2. The concept of affinity chromatography preparations has been technically available for protein separation and purification, and improved upon over the past 30 years, Ey, P.L., et al., Isolation of pure IgG_I, IgG_{2a}, and IgG_{2b}. immunoglobulins from mouse serum using protein A-Sepharose, *Immunochemistry* 1978;15:429-436. This type of device represents one of the few new examples of linking *in vivo* production of TNF inhibitors to *in vitro* removal and return of the purified extracted plasma to the patient to prevent fluid reduction.

5. The patient is a 55 year old Russian gentleman with metastatic melanoma. The patient smoked 2-3 packs of cigarettes a day for some 20 years. He quit this habit several years ago. He was also a heavy alcohol user in years past but had decreased his intake to 1-2 glasses of wine a day. Review of his medications on this date revealed methylprednisolone 4 mg in AM and 4 mg in PM. Apparently this was being taken as replacement therapy for adrenal cortical suppression that was graded iatrogenically at the time of the treatment of his alveolitis (see below). He was additionally taking narcotic analgesics. As a child he suffered the usual childhood diseases, denies rheumatic fever, scarlet fever or diphtheria. As an adult he has had no major medical illnesses save those

described above. He has had no other major surgeries in the past and has no known allergies.

His history of present illness began in November of 1995 when he noted growth of a right facial naevus which bled and enlarged over the period of one year. This was treated initially by cryotherapy. It regrew within two months and was excised. Histology was that of a malignant melanoma (Clark's level unknown). Staging work up at the time was negative and included CT scans of the head, neck, chest and abdomen. He remained disease free until March of 1996 when he developed right cervical and right submental adenopathy. Preoperative CT scan of the head, neck, chest and abdomen confirmed the right cervical adenopathy but revealed no other sites of metastases. In June of 1996 he underwent re-excision with a right radical neck dissection. In this material, one lymph node was histologically confirmed to involve melanoma. The patient was treated with a course of Vindesine 3 mg/m^2 every three weeks, Dacarbazine 100 mg/m^2 every three weeks for four cycles. He subsequently developed cutaneous metastases in the skin of his left shoulder, multiple metastases to the scars within the left anterolateral neck and multiple axillary metastases treated with fifteen subsequent excisions of recurrent metastases. In March of 1999 he was offered a trial of Interleukin-2 but on this developed severe pulmonary toxicity that had a protracted course and was diagnosed as idiopathic fibrosing alveolitis. Interleukin-2 was discontinued and he received radiation therapy to his right neck and axilla for six weeks beginning in the month of May 1999. He developed low back pain in August of 1999. Work up in October of 1999 revealed bone metastasis of the vertebral body of T-11 and subsequent MRI revealed a lytic destructive process in the right transverse process and pedicle of the 11^{th} thoracic vertebra, as well as complete replacement of the vertebral body at T-11. Additional metastases were appreciated in the vertebral body of the 9^{th} thoracic vertebral as well as the 10^{th}. Also there was involvement of L-1 and L-2 vertebral bodies. Tumor seen again on the March 16, 2000 MRI revealed growth posteriorly from the mid body of the 11^{th} thoracic vertebral into the spinal canal by 7.4 to 7.8 mm with posterior displacement of the spinal cord. CT scan of the chest, abdomen and pelvis revealed possible multiple liver metastases but no other suggestion of visceral metastases.

6. The patient was then considered for a trial of UltraPheresis™ in an effort to reduce solubilized receptors to tumor necrosis factor, both sTNF-R1 and sTNF-R2. As facilities for the application of this form of semi-selective plasma exchange did not exist in Moscow at this time, affinity column separation of inhibitors was explored. Monoclonal antibodies against sTNF-R1 and R2 delivered to the Cardiology Research Center in Moscow for Dr. Sergei N. Petrovsky, PhD, head of the group for Affinity Sorbents for Medicine, Pocard, Ltd, 3-rd Cherepkovskaya str., 15a, Moscow, 121552, Russia. Ninety milligrams of anti sTNF-R1 monoclonal antibody and 180 mg of anti sTNF-R2 monoclonal antibody were then bound with sterile Sepharose® using cyanogen bromide in a glass column previously described for use in the lipopack cholesterol absorbent column technology. The particular methodology used is well described and is commercially available in Russia for the development of these LDL absorbent columns. The columns were prepared under sterile conditions in a GSIO 9,001 facility. They were subjected to endotoxin testing, viral, fungal and bacterial cultures, and prepared for human use under written Informed Consent and under approval of the Kremlin President's Hospital Medical Center.

7. On May 2nd, 2000 the patient's physical examination was that of a well-developed, well-nourished male who appeared his stated years. Examination of his head revealed a normal hair distribution and texture. His tympanic membranes and external auditory canals were clear. The sclerae and conjunctivae were clear. The pupils were round, reactive to light and accommodation. EOM intact. Funduscopic examination was normal. He had a healed graft over his right inferior cheek and extensive scarring over the right anterolateral neck consistent with his history of prior right radical neck dissection. There were no demonstrable pathologic masses within the skin, the scar, or pathologic nodes appreciated either in the cervical nodes or the supraclavicular fossae bilaterally. His lungs were clear to ausculation and percussion. His precordium demonstrated a non-displaced PMI, a normal S1 and S2 without gallop, murmur or rub. With the right arm exhibited there was 3+lymphedema. The right axilla was poorly examined due to extensive scarring in that area but no palpable nodes were appreciated. His abdomen was mildly obese. His liver and spleen were normal to physical examination. His axillary lymphatics were unremarkable. The genitalia was that of a

normal mature male without pathologic mass. The lower extremities revealed no edema, cyanosis or clubbing and exhibited full ROM. His neurologic examination included a normal mental status. Cranial nerves 2-12 were intact. His DTR's were 2+ and symmetric. Motor and sensory testing was normal. His cerebellar examination revealed no dysmetria, dysarthria or dysdiadochokinesia. He was essentially confined to bed due to back pain only, but was able to roll from left to right without assistance. He had been confined to a wheelchair for the antecedent two months due to back pain and was wearing a back brace which was removed for physical exam.

His laboratory parameters included a hemoglobin of 8.8 gms, WBC 2,800 with normal differential. His platelet count was 121,000. The comprehensive metabolic panel was unremarkable and alkaline phosphatase was normal.

An MRI scan of the patient's 11th thoracic vertebral body revealed a mass placing pressure on the spinal cord. This was taken during the week prior to intensive therapy started in April of 2000 and continuing through May.

8. On the first day an 18 gauge plastic cannula was inserted in the left antecubital vein. A second was established in the right greater saphenous vein of the leg. The patient was connected to a standard Cobe Spectra centrifically based plasma separator. Six hundred cc's of plasma was then harvested and replaced with 5% albumin in saline. The patient's plasma was then pumped over column one which contained 45 mg of anti sTNF-R1 monoclonal antibody and then passed to column two which contained 90 mg of anti sTNF-R2 monoclonal antibody. The material eluted from the column was then analyzed for the level of each inhibitor still in the plasma and 50 cc's of that plasma was then injected into the patient at the end of pheresis to look for any febrile reactions or allergic reactions. He tolerated this with no apparent clinical adverse effect.

Subsequent analyses of the patient's plasma and the eluate of the column revealed that the column was able to capture essentially all of the inhibitor presented to it in this 600 ml plasma volume. The patient was maintained in the hospital over night and on the morning of the 4th of May, he was brought from hospital room back to the apheresis suite. He had a comfortable evening and ate a normal dinner and breakfast. The IV's were re-established in the same sites. The patient was re-attached to the Cobe Spectra

machine and on this date, 3 liters of plasma was harvested and delivered to the columns as described above in a continuous fashion until 3 liters of plasma was treated.

His R1 level before treatment was 1500 and after treatment was 1450. His R2 level before treatment was 5000 and after treatment was 3800 on this date. Again he tolerated the procedure well with no clinical adverse effect and no increase in pain in his back.

On the third day the 6th of May, the treatment was repeated. We again pheresed 3 liters of plasma over the columns in an identical fashion as described above. His pretreatment R1 was 2300, post treatment R1 was 1600. Pretreatment R2 was 5200, post treatment R2 was 3200. At the end of each treatment the columns were washed with glycine buffer at a pH of 2.5 to elute the bound inhibitor from them and measure them quantitatively. It was determined that at these amounts of treated plasma the columns were not saturated and significant quantities of inhibitor removed.

His 4th treatment was on the 7th of May. He was increased to 4 liters of treated plasma. The procedures were repeated each day with gradual escalations in amount of plasma treated to a maximum treated plasma of 8 liters on the May 10th, 11th, 12th, 13th, and 14th. On May 16th we used two columns in parallel thus increasing the amount of plasma delivered to each column remained at 30 ml's per minute but using two columns we were able to treat a total of 60 ml's of plasma per minute. This resulted in a pretreatment R1 of 2600 and a post treatment of 1700. R2 pretreatment was 4250 and went to post treatment of 2700.

He was subsequently treated with 8 liters of plasma a day using the double column method. On the 21st of May he had a repeat CAT scan of his spine which revealed complete resolution of tumor. Three days after that, May 24th,he had a repeat MRI which was compared to the pretreatment MRI and confirmed a complete response. The patient was followed carefully in the hospital by his attending physicians as well as attending neurosurgeons, who followed him on a daily basis concerned about tumor bleeding or tumor swelling in his tight and anatomically dangerous place but fortunately the patient enjoyed a complete response with no apparent adverse effect.

For the details of daily treatment in terms of volumes, columns, blood flow rates and plasma flow rates see Table 1.

9. The patient has enjoyed an apparent complete response without any significant adverse effect. He was able to get up and walk after the 4th procedure. We are planning an two additional courses in an endeavor to consolidate this response. This case, we feel, is consistent with our previous observations that a salutatory tumor response can be achieved in melanoma by removing solubilized receptors to TNF. This is the first time that we have been able to remove only the solubilized receptors to TNF and essentially eliminate the possibility that it is something in normal replacement solution that is being facilitory to this response rather than simply the removal of something. This column is so specific that it removes only sTNF- R1 and R2 and that is the only explanation for the response that this man has had from an oncologic point of view. A profound column yield was observed on the third treatment day for sTNF-R2 with modulation for the remaining treatment days throughout this fifteen day course. R1 peaked on treatment day 7 with the total amount removed of 6 million pg. This also modulated throughout the course of treatments but never approached the 16 million mark set by sTNF-R2.

Radiographic examination on the day following his first fifteen day course of apheresis with anti R1 and anti R2 affinity column extraction revealed no melanoma and considerable reduction of the lesion at the 4th lumbar vertebral body. Currently the patient remains active, with good appetite, is walking normally and his back pain is much improved. He has positive anticipation for his second course of apheretic treatments.

10. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: _____ _____

M. Rigdon Lentz

TABLE 1

Review of TNF-R on Tenth Day of Treatment
(Concentration of each inhibitor each hour of treatment)

	R-1	R-2
Start	2569	4250
after 1.2 liter sample taken in live pre-column	739	1309
after 2 liter	393	421
after 3 liter	467	728
after 4 liter	514	810
after 5 liter	356	575
after 6 liter	481	906
after 7 liter	529	871
after 8 liter	529	771
immediately after treatment	1659	2679

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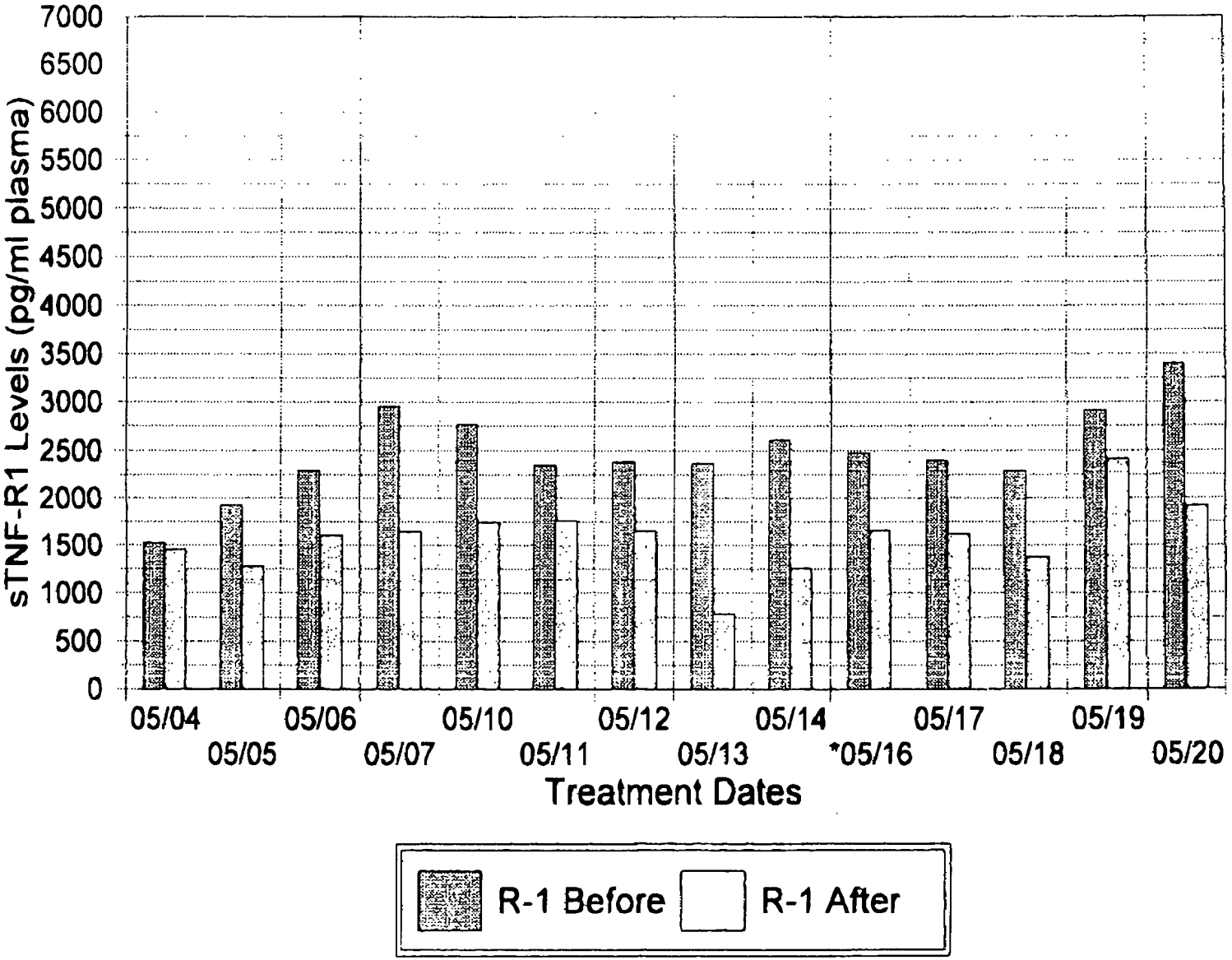
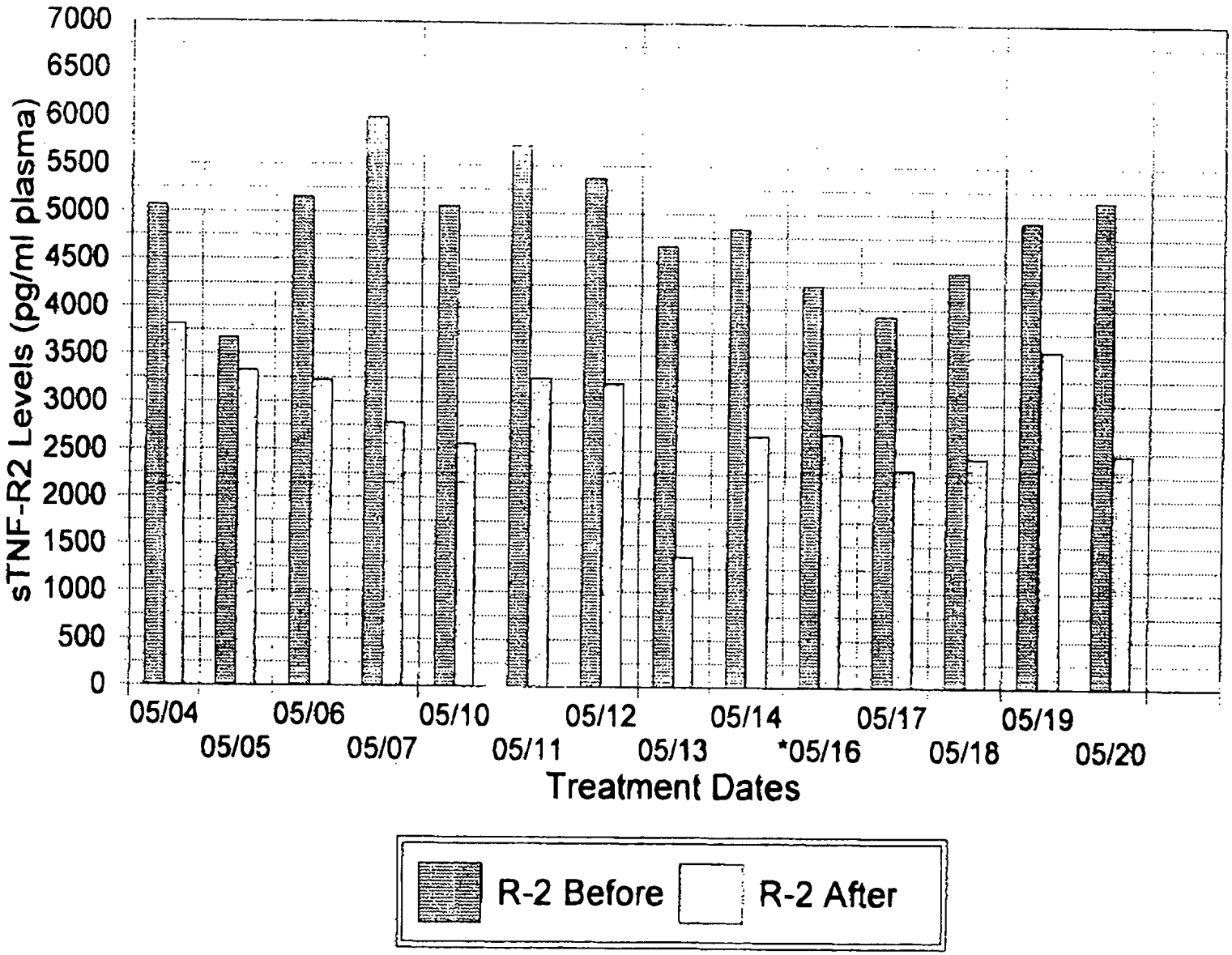
Figure 2A



* From 5/16/00 thru 5/20/2000 double columns were connected in parallel to reduce pheresis time.

Figure 2B



* From 5/16/00 thru 5/20/00 double columns were connected in parallel to reduce pheresis time.

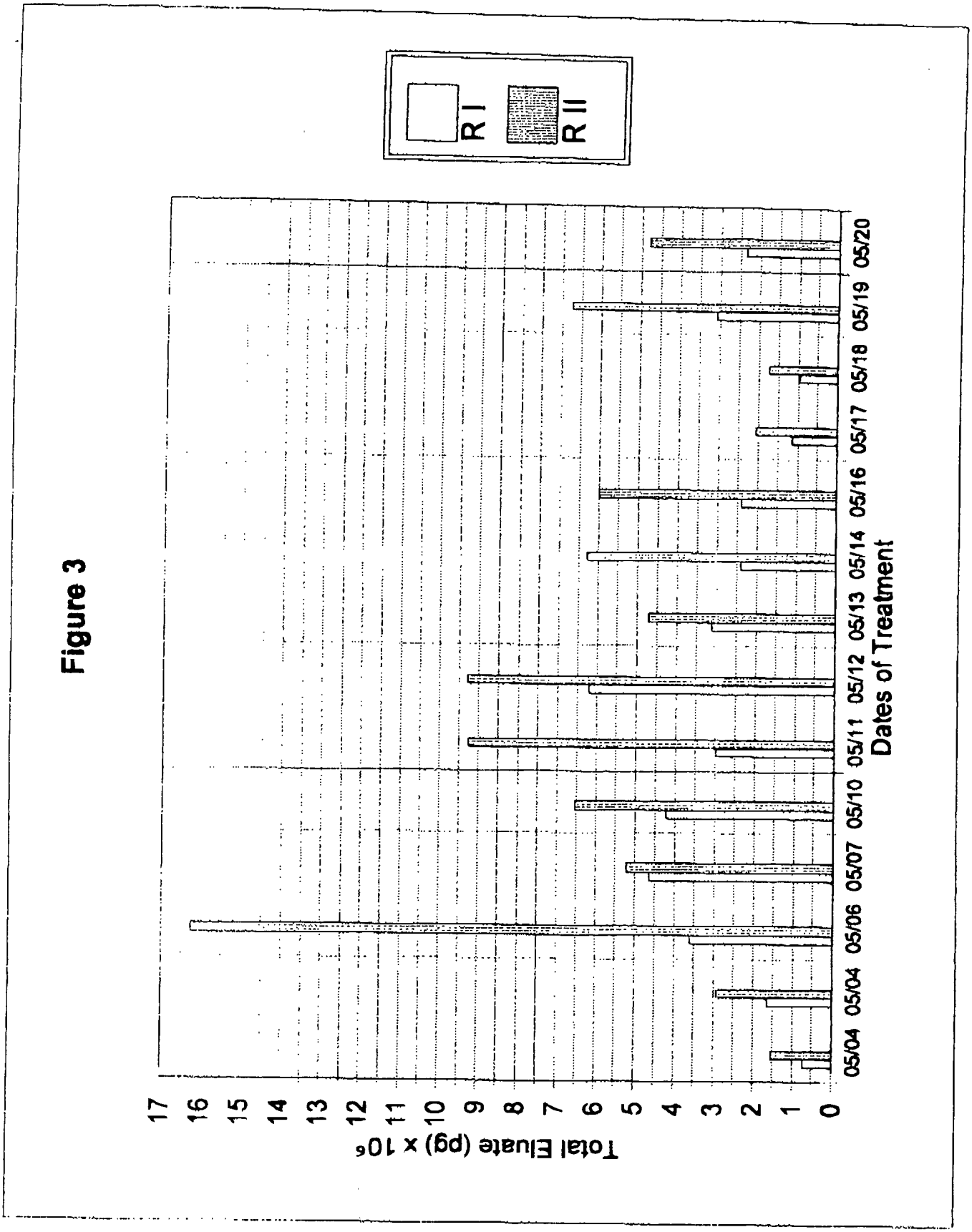

R I
R II
Total Eluate (pg) x 10⁶
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
05/04
05/04
05/06
05/07
05/10
05/11
05/12
05/13
05/14
05/16
05/17
05/18
05/19
05/20
Dates of Treatment

Figure 3